

Toxicity of MXD-100 to the Mollusk *Physella acuta* (Draparnaud, 1805) and the freshwater shrimp *Macrobrachium cf. olfersii* (Wiegmann, 1836)

Abstract

Limnoperna fortunei,¹ popularly known as the golden mussel, is an invasive species widely distributed in Brazil that has affected hydroelectric plants due to the fouling process of pipes and equipment. As an emergency response, the plants use the anti-fouling product MXD-100. This study evaluated the self-purification and acute toxicity of MXD-100 at different concentrations on the mortality of the freshwater shrimp *Macrobrachium cf. olfersii*² and the mollusk *Physella acuta*,³ species with a wide distribution in Brazil. The analyses were divided into four experiments, which had the following objectives: (1) to monitor the lethality of MXD-100 for *Physella acuta*; (2) to analyze the possible self-purification of MXD-100 with *Macrobrachium cf. olfersii*; (3) to evaluate the residual effect of the molluscicide with *Physella acuta*; and (4) to analyze eventual self-purification and possible toxic effects of the product on *Macrobrachium cf. olfersii*. The water quality parameters evaluated were: temperature, pH, electrical conductivity, total dissolved solids, chlorine, alkalinity, ammoniacal nitrogen, dissolved oxygen, residual chlorine, and turbidity. Six aquariums were used to carry out the experiments, one as a control and the other five receiving, individually, the following concentrations of MXD-100: 0.042 mL L⁻¹, 0.083 mL L⁻¹, 0.125 mL L⁻¹, 0.167 mL L⁻¹, and 0.208 mL L⁻¹. Our results demonstrated the persistence of the deleterious and toxic effects of MXD-100 on the target species studied, highlighting the need to reassess the use of this molluscicide in aquatic systems, prioritizing alternatives with less environmental impact.

Keywords: aquatic ecotoxicology, emerging contaminants, aquatic bioassay.

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Introduction

The impacts of changes to aquatic ecosystems, caused by different anthropogenic and natural factors, such as the removal of riparian vegetation and wetlands; the construction of dams and the introduction of exotic species, trigger effects such as eutrophication; reduction or increase in navigation and river transport; changes in the load of suspended materials and interference in the hydrological regime. These effects, together, affect the biodiversity and ecological balance of aquatic environments.⁴

Biological invasion is the act or effect of one or more organisms invading and establishing themselves in environments where there were no previous records of the species.⁵ They represent a major threat to the integrity of the biological communities of an ecosystem. Factors such as food availability, environmental conditions, the number of individuals introduced, and the absence of predators are relevant variables in this process.⁶ In a scenario favorable to the bioinvasion process, the species *Limnoperna fortunei*,⁷ popularly known in Brazil as the golden mussel, stands out. This is a freshwater bivalve mollusk of the family Mytilidae Rafinesque, 1815, which is mostly represented by mussels that inhabit oceans and estuaries.⁵

The phylum Mollusca currently comprises more than 250,000 species, constituting, together with the Crustacea, the main groups of invertebrates,⁸ after Hexapoda. The classes Gastropoda and Bivalvia are the main ones in freshwater ecosystems. Some species have high economic value in animal and human food, some are used in jewelry production, while others have medical and veterinary importance.^{5,9} There are exotic mollusks established and adapted in Brazilian territory, these can be divided into three groups: Group I - free-living

species; Group II - species kept in captivity in Brazil; and Group III - potential invaders. *Physella acuta*³ falls into the first group, being fully adapted to various Brazilian ecosystems. Considering biological variables of the species such as biotic potential and reproductive strategy, populations grow, even in the presence of predators and parasites.¹⁰

The golden mussel originates from Southeast Asia and is believed to have been introduced to South America around 1991 via ship ballast water, gradually colonizing aquatic environments in Argentina, Uruguay, and Brazil.¹¹ This species has a high invasive potential, feeds by filtering algae and organic debris, and is versatile in many environments.¹² Considering its rapid proliferation, it becomes a global threat demanding attention, given that aquatic transport favors invasion through biofouling and the use of ballast water.^{3,9}

The damage caused by encrustations of this bivalve has become a difficult problem to solve for companies that depend on water intake from water sources to supply their production processes or for energy generation (Camargo et al., 2021c).¹ This mollusk has also been responsible for losses by encrusting itself on the hulls of vessels, net cages, and populating a large part of the surface of hydroelectric dam reservoirs.^{7,13}

To reduce infestation in facilities, one population control measure adopted is the use of chemical products, usually classified as oxidizing and non-oxidizing biocides, which can be applied to the network from a single point.^{14,15} An example of a widely used Brazilian commercial biocide is MXD-100, which is an anti-fouling agent with active compounds based on tannin extracts and quaternary ammonium compounds.

Macrobrachium olfersii is a shrimp species in the family Palaemonidae, with a wide geographic distribution, including South America and Brazil.^{16–18} Due to its biological characteristics and meat, it is widely used in aquaculture.¹⁹ It is a predominantly freshwater species, which, according to some authors, requires brackish water to complete its life cycle, mainly for reproduction and larval metamorphosis.^{20,21} It exhibits sexual dimorphism, with males being larger and having large chelipeds with bristles and spines; while females are smaller.¹⁶ Both have a translucent carapace with variable coloration. The presence of variable characters in the genus *Macrobrachium* makes the correct identification of species uncertain,^{2,22} and due to phenotypic plasticity, it has several synonyms.²³

This study aimed to evaluate the acute toxicity of MXD-100 at different concentrations regarding the mortality of the freshwater shrimp *Macrobrachium cf. olfersii* and the mollusk *Physella acuta*. The analyses were divided into four experiments. The objective of each experiment was: (1) to monitor the lethality of MXD-100 to *Physella acuta*; (2) to analyze the possible self-purification of MXD-100 with *Macrobrachium cf. olfersii*; (3) to evaluate the residual effect of the molluscicide on mollusks of the species *Physella acuta*; and (4) to analyze the eventual self-purification and possible toxic effects of the product on *Macrobrachium cf. olfersii*.

Material and methods

The experiment was conducted in the Nico Nieser laboratory of the Ecology & Evolution discipline at the Institute of Biological and Natural Sciences, located at the Federal University of Triângulo Mineiro, in the municipality of Uberaba, Minas Gerais, Brazil. Six aquariums were used, each with a useful volume of 20 liters. The setup included river gravel, with an approximate granulometry of 8 mm, to increase the structural diversity of the habitat, in addition to a piece of common clay brick, used in civil construction. Each aquarium was equipped with a submersible motor pump to ensure aeration of the system and received 12 liters of previously dechlorinated potable water. Figure 1 illustrates the experimental units used in the experiments.

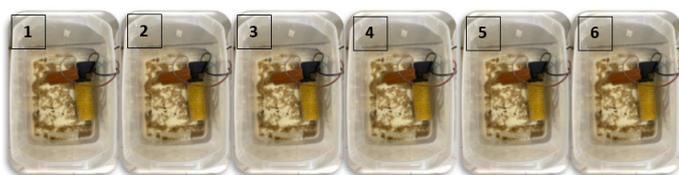


Figure 1 Aquariums used as experimental units in toxicity tests using the freshwater shrimp *Macrobrachium cf. olfersii* (Wiegmann, 1836) and the mollusk *Physella acuta* (Draparnaud, 1805).

The water quality parameters evaluated during the experiments were: temperature, pH, electrical conductivity, total dissolved solids (TDS), total alkalinity, ammoniacal nitrogen, dissolved oxygen (DO), residual chlorine, and turbidity. Alkalinity was quantified using the Nautilus® brand test strip method. For quantification of ammoniacal nitrogen, the LabconTest® NH3 rapid test was used. The parameters temperature, pH, dissolved oxygen (DO), electrical conductivity, turbidity, and total dissolved solids (TDS) were monitored and quantified using an electrometric method with the Horiba® U-52 probe.

To choose the concentrations of MXD-100 to be used in the experiments, the volume of the aquariums, the dosages recommended by the manufacturer, and also those used in the methodologies described by Ribeiro and Pelli;²⁴ Montresor et al.²⁵ and Moreira et al.⁶

were considered. Thus, considering the volume of 12 liters and the dosages recommended by the manufacturer of 0.5 mL, 1.0 mL, 1.5 mL, 2.0 mL and 2.5 mL, the following concentrations of MXD-100 were used: 0.042 mL L⁻¹, 0.083 mL L⁻¹, 0.125 mL L⁻¹, 0.167 mL L⁻¹ and 0.208 mL L⁻¹, in aquariums 2, 3, 4, 5 and 6, respectively. Aquarium 1 was the control and did not receive any dosages of the molluscicide. The experimental design adopted used few aquariums and animals due to budgetary limitations.

First experiment

On the first day of the experiment, the aquariums were set up and nine individuals of the species *Physella acuta* were added to each aquarium. Figure 2 illustrates the *Physella acuta* snail used in the experiment. On the second day of the experiment, physicochemical analyses of the water were performed, and on the third day, 50 mL of a saturated calcium carbonate solution was added to raise the water's pH. An alkaline pH favors the integrity of the snail shells, which are mainly composed of calcium carbonate. Therefore, adjusting the pH ensured suitable conditions for maintaining the animals' well-being. On the same day, MXD-100 was added to the aquariums at the concentrations described previously. During the experiment, the eventual mortality of the snails was monitored, and physicochemical analyses of the water were performed. On the eighth day of the experiment, the snails were counted and removed from all aquariums. The mollusks' death was confirmed when their bodies were exposed outside their shells; soft; insensitive to touch; had lost their original color, showing signs of decomposition.



Figure 2 The snail *Physella acuta* (Draparnaud, 1805) used in the experiment, with an estimated total length of 8 mm.

Second experiment

The second experiment aimed to analyze the possible self-purification of the MXD-100, through the activation of the filtration systems and verified by means of the eventual mortality of the freshwater shrimp species *Macrobrachium cf. olfersii* (Wiegmann, 1836). The filtration systems of all aquariums were activated on the ninth day of the experiment and remained in operation for four days, aiming to promote the oxidation of the medium. After this period, on the 13th day of the experiment, a shrimp was introduced into each aquarium, previously subjected to an acclimatization process, where they remained for four days, until the 17th day of the experiment. Figure 3 illustrates specimens of the freshwater shrimp species *Macrobrachium cf. olfersii*. The shrimp's death was confirmed when it stopped responding to stimuli, losing its original position, from a straight and stretched posture to a curved C-shape. Its long antennae remained immobile; and its body tended to change color, turning opaque and yellowish, showing signs of decomposition. Figures 4A and 4C show a live animal, and Figure 3 shows dead specimens.



Figure 3 Specimens used in these experiments of the freshwater shrimp species *Macrobrachium cf. olfersii* (Wiegmann, 1836).

Figure 4 illustrates the acclimation process for transferring shrimp to the experimental aquarium. For acclimation, shrimp previously acclimated in a vivarium were placed in plastic bags containing 1 liter of water from their original aquarium. Each bag was then kept in the experimental aquarium for approximately 15 minutes, allowing for gradual adaptation to the new conditions and preventing thermal shock. During the experiment, analyses of physicochemical parameters were performed. The shrimp were fed on alternate days with fish food from the manufacturer TetraFin – Goldfish Flakes®. On the 17th day of the experiment, the mortality rate was verified and new analyses of the physicochemical parameters of the water were performed.



Figure 4 Acclimation process for transferring freshwater shrimp of the species *Macrobrachium cf. olfersii* (Wiegmann, 1836) to the experimental aquarium: (A) vivarium aquarium; (B) acclimation in the experimental aquarium; and (C) shrimp released into the experimental aquarium.

Third experiment

The third experiment began on the 17th day, with the introduction of *Physella acuta* snails into the aquariums, immediately after the shrimp were removed, without making any changes to the water. The objective of this experiment was to evaluate the residual effect of the molluscicide on the snails. For this purpose, six snails were introduced into the control aquarium, three snails into aquariums 2, 3, and 4, and four snails into aquariums 5 and 6. The physicochemical analyses of the water and the mortality of the snails were monitored daily until the 27th day of the experiment. At the end of the period, the snails were removed from the aquariums.

Fourth experiment

The objective of the fourth experiment was to analyze the eventual self-purification and possible toxic effects of MXD-100 on freshwater shrimp of the species *Macrobrachium cf. olfersii*. The analyses of the physicochemical parameters of the water and the monitoring of shrimp mortality were verified on the day of the molluscicide dissolution in the aquariums and one week later. The experiment began on the 30th

day, adding 2 liters of water from the shrimp's original aquarium to each experimental tank to compensate for volume losses caused by transfer and evaporation. Then, one shrimp was introduced into each tank, which had been previously acclimatized. On the 31st day of the experiment, MXD-100 was diluted in the tanks at the described concentrations, and physicochemical analyses of the water were performed. On the 38th day, the last day of the experiment, the shrimp were removed from the tanks. Mortality was used as a parameter to evaluate the toxicity of MXD-100 at different dosages, considering possible self-purification and residual toxic effects of the molluscicide. The experimental design adopted in this study is summarized in Figure 5. Statistical analysis was performed using the chi-square test, a non-parametric inferential statistical tool, to verify the potential effect of the molluscicide on the target organisms.

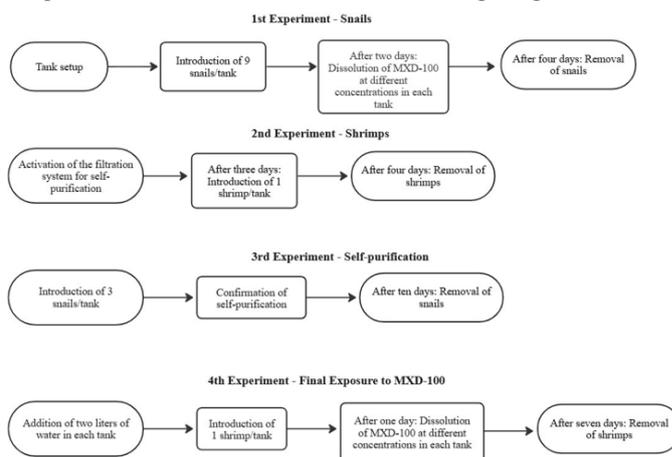


Figure 5 Flowchart of the experimental design adopted in the experiments.

Results and discussion

First experiment

The physicochemical parameters of the water on the first day of the experiment were similar in all aquariums, indicating uniformity in the initial environmental conditions. In all experimental units, the pH found was 6.8 and the alkalinity showed values of 40 mg L⁻¹ of CaCO₃. No levels of ammonia (N-NH₃) were detected and the residual chlorine showed values lower than 0.5 mg L⁻¹, characterizing water within the expected standards. These results confirm that the initial conditions were adequate for the maintenance of the test organisms before the addition of the molluscicide MXD-100.

The concentration of hydrogen ions in solution exerts a direct influence on most chemical constituents, therefore being a fundamental parameter in the evaluation of the quality of natural waters. The range of this parameter considered suitable for most aquatic life is quite narrow and critical, ranging between 6 and 9. Thus, the value of 6.8 found in all experimental units is within this range, ensuring appropriate conditions for the test organisms (Ferreira et al., 2024).

Alkalinity is the ability of a body of water to neutralize acids, a process known as the buffering effect. It is a parameter commonly used to describe the quality of surface and groundwater (Ferreira et al., 2024). The alkalinity of 40 mg L⁻¹ of CaCO₃ found in all aquariums indicates a moderate buffering capacity. This means that the water has a sufficient amount of bicarbonate, carbonate, or hydroxide ions to resist variations in pH. This stability is important, especially in environments where abrupt changes can stress or even harm aquatic organisms.

Chlorine is the most widely used disinfectant in water purification, presenting an easily determined residual effect that is not harmful to humans and tends to zero naturally. However, for sensitive aquatic organisms, the absence of chlorine is the ideal condition, as it eliminates the risk of stress associated with this element, which can be harmful even at low concentrations.²⁶ In this sense, the values below 0.5 mg L⁻¹ (which corresponds to the detection limit of the method) found in all experimental units indicate an environment suitable for the development of the organisms studied.

Toxic ammonia levels were not detected by the method used. The values are consistent with the water source and intended use. On the second day of the experiment, the physicochemical parameters of the water between the aquariums showed similar values. The average temperature was 22.25 ± 0.16 °C, the average electrical conductivity was 241.33 ± 6.86 µS/cm, and the turbidity was 22.85 ± 3.49 NTU. The average value of total dissolved solids (TDS) was 157 ± 0.005 mg L⁻¹, while dissolved oxygen had an average of 9.76 ± 0.66 mg L⁻¹. For residual chlorine and ammoniacal nitrogen (N-NH₃), the values remained at 0 mg L⁻¹, as did the alkalinity, which remained constant at 40 mg L⁻¹ of CaCO₃ in all aquariums.

Regarding pH, the values ranged around 5.77 ± 0.30. This result indicates that there was a significant drop in pH in all aquariums when compared to the previous day. This pH level is more acidic and is below the recommended limit for many aquatic organisms, especially those with calcareous structures, such as the shells of mollusks and the exoskeleton of crustaceans. According to Siegel et al.,²⁷ the exoskeleton of crustaceans, such as crabs and shrimp, has alkaline characteristics. This property is mainly due to its chemical composition. The main component of the exoskeleton of arthropods in general is chitin, a polysaccharide. However, in crustaceans, the exoskeleton is significantly hardened by the impregnation of mineral salts, with calcium carbonate (CaCO₃) being the most prominent.²⁸ Calcium carbonate is a salt that, in aqueous solution, confers an alkaline (basic) pH due to the hydrolysis of the carbonate ion. This substance is known to react with acids. The significant presence of calcium carbonate in the carapace of crustaceans is what gives it rigidity and also its alkaline properties.^{29,30}

Studies conducted by Xu et al.³¹ analyzed the effects of acidification on bivalve shells and found that acidic pH reflects a decrease in shell hardness and an increase in the porosity of calcareous structures. Temperatures ranged from 22.1 °C to 22.5 °C, which is considered adequate for the organisms studied.³² Temperature is a parameter that affects the physical and chemical properties of water and directly interferes with animal welfare. The feeding, reproductive habits and metabolic rates of organisms also undergo changes resulting from temperature variations. In addition, with increasing temperature, the amount of dissolved oxygen decreases.³³⁻³⁵

Dissolved oxygen values ranged from 8.76 to 10.38 mg/L. These values are acceptable for most aquatic organisms, ensuring that there is no stress.³⁶ No snail mortality was recorded, suggesting that environmental conditions were compatible with animal welfare and within acceptable limits. The results of the physicochemical analyses of the aquarium water on the third day of the experiment, before the addition of the saturated calcium carbonate solution and MXD-100, indicated an average temperature of 22.38 ± 0.24 °C, slightly acidic pH in all experimental units, and a significant reduction in turbidity compared to the previous day. No snail mortality was observed either, suggesting that the organisms are tolerating the environmental conditions.

Although water quality parameters were monitored to verify whether the conditions in each aquarium were ideal for receiving the snails, once established, *P. acuta* can adapt to a wide range of habitat conditions, including polluted freshwater. Additionally, the species is highly tolerant to high temperatures and can successfully withstand extreme values of physical and chemical parameters, possibly the key to its biological success.^{37,38} Table 1 presents the results of the physicochemical analyses of the aquarium water on the third day of the experiment, immediately after the addition of the saturated calcium carbonate solution and MXD-100.

Table 1 Physicochemical parameters of the aquarium water on the third day of the experiment, immediately after the addition of the saturated calcium carbonate solution and MXD-100

Parameter	Aquarium					
	1	2	3	4	5	6
MXD-100 Conc.	0	0.042	0.083	0.125	0.167	0.208
Temperature	22.99	22.42	22.19	22.07	22.46	22.55
pH	8.06	8.19	8.16	8.19	8.69	8.38
Electrical Conductivity	323	299	277	292	375	296
Turbidity	47	28.9	19.3	17.2	24	30.5
TDS	210	194	180	190	244	192
DO	9.88	8.4	8.02	7.8	7.86	7.56

Note: MXD-100 conc. (mL L⁻¹); temperature (°C), electrical conductivity (µS/cm), turbidity (NTU), TDS (mg L⁻¹) and DO (mg L⁻¹)

The pH results show that the addition of the saturated calcium carbonate solution was sufficient to raise the pH of the experimental units. The materials used for liming raise both the pH of the water and the total alkalinity and total hardness. No snail deaths were observed after the dissolution of MXD-100 in the aquariums, indicating that the toxic effect of the biocide occurs gradually. Table 2 presents the mortality of *Physella acuta* and the results of the physicochemical analyses of the water in the aquariums on the eighth day of the experiment, five days after the introduction of MXD-100.

Table 2 Physicochemical parameters of the aquarium water and record of mortality of *Physella acuta* (Draparnaud, 1805) on the eighth day of the experiment, five days after the introduction of MXD-100

Parameter	Aquarium					
	1	2	3	4	5	6
MXD-100	0	0.042	0.083	0.125	0.167	0.208
Temperature	21.96	21.3	20.94	20.94	20.72	21.26
pH	6.61	6.64	6.63	6.85	6.71	6.85
Electrical conductivity	405	410	413	397	450	410
Turbidity	18.4	13.5	17	33.5	55.9	56.6
TDS	263	267	267	258	292	267
DO	6.94	6.14	5.41	5.13	6.14	4.39
Mortality	0	9	9	9	9	9

Note: conc. MXD-100 (mL L⁻¹); temperatura (°C), condutividade elétrica (µS/cm), turbidez (NTU), TDS (mg L⁻¹) e OD (mg L⁻¹)

The data presented in Table 2 show the mortality of all snails from all experimental units, except in the control aquarium, which did not receive dosages of the molluscicide. It is evident that even the lowest concentration of MXD-100 used, of 0.042 mL L⁻¹, was sufficient to cause the mortality of all snails. The chi-square analysis indicated significance at the 0.01% level. Similar studies carried out by Moreira et al.⁶ investigated the effects of two biocides, MXD-

100 and Sodium Dichloroisocyanurate (NaDCC), by evaluating morphological changes and gene expression in the gills of the golden mussel, *Limnoperna fortunei*. Both biocides were able to modulate the expression of defensive genes and morphological changes. While NaDCC required continuous exposure to control golden mussel infestations, MXD-100 caused severe damage to these organisms in just 24 hours.

Second experiment

Table 3 presents the results of the physicochemical parameters of the water in the monitored aquariums on days 13 and 17 of the experiment. Despite the decrease in dissolved oxygen (DO), no

mortality of *Macrobrachium cf. olfersii* was observed, indicating that the filtration systems favored self-purification and maintained water quality at levels compatible with the survival of the species. The other physicochemical parameters remained stable between the days evaluated, indicating stability of environmental conditions in the aquariums. The temperature remained between 23 and 25 °C and the pH close to neutrality, conditions suitable for the development of the species *Macrobrachium cf. Olfersii*.³² Conductivity, total dissolved solids, and alkalinity showed little variation, evidencing ionic stability and good buffering capacity. The low levels of non-ionized ammonia indicate that the filtration and aeration system was efficient and did not constitute a limiting factor in maintaining aquatic life.

Table 3 Physicochemical parameters of the water in the experimental aquariums, on the 13th and 17th day of the experiment. Parameters exposed to MXD-100 and self-purification verified through the mortality of shrimp of the species *Macrobrachium cf. olfersii* (Wiegmann, 1836)

Parameter	Aquarium											
	1		2		3		4		5		6	
Day	13°	17°	13°	17°	13°	17°	13°	17°	13°	17°	13°	17°
Temp.	23.9	25.1	23.6	24.5	23.4	24.6	23.1	24.4	23.1	24.5	23.2	24.5
pH	7.24	6.88	7.23	7.02	7.24	7.18	7.14	7.24	7.15	7.2	7.01	7.13
E.Con.	461	471	473	490	457	477	459	480	509	522	433	463
Tur.	0	0	0	0	0	0.5	0	0	0	0	0	0
TDS	300	307	307	318	297	310	298	312	325	334	279	300
Alk.	120	120	120	120	120	120	120	120	120	120	120	120
NH3	0	0	0	0.5	0	0.5	0	0.25	0	0.25	0	0.25
DO	6.6	5.13	6.22	5.32	6	4.69	6.92	4.87	5.9	3.92	6.7	4.34

Note: temperature (°C), electrical conductivity (µs/cm), turbidity (NTU), TDS (mg L⁻¹), alkalinity (mg L⁻¹ of CaCO₃), NH3 (mg L⁻¹) and DO (mg L⁻¹)

Third experiment

The physicochemical parameters of the water in the monitored aquariums on the 17th and 27th days of the experiment were adequate for the animals: *Physella acuta*, which was exposed to the residual effect of MXD-100. The parameters monitored were temperature, electrical conductivity, turbidity, total dissolved solids, alkalinity, ammoniacal nitrogen, and dissolved oxygen. Considering the survival of all experimental animals, the authors believe that the system showed self-purification capacity. The introduction of organic matter into water bodies results in a reduction in the concentration of dissolved oxygen.³⁹ Purely physicochemical processes also influence the dissolved oxygen balance, and chemical reactions can eventually consume dissolved oxygen in the medium.⁴⁰ The other physicochemical parameters did not show significant variations between the days evaluated, indicating the stability of the system. Temperature and pH remained within ranges compatible with freshwater organisms. Electrical conductivity and total dissolved solids fluctuated slightly, reflecting ionic balance. Alkalinity remained constant, demonstrating good buffering capacity. Low concentrations of non-ionized ammonia confirm the absence of toxicity, corroborating the efficiency of the MXD-100 self-purification promoted by the system.

Fourth experiment

The results of the physicochemical parameters of the water in the experimental aquariums on the 31st day of the experiment, immediately after MXD-100 was diluted in the experimental units, revealed that no immediate effects occurred on either the physicochemical parameters of the water or the experimental organisms. The absence of immediate shrimp deaths on the day MXD-100 was dissolved in the aquariums can be explained by the gradual nature of the product's action. The molluscicide used may not have an immediate effect due

to its mechanism of action, where, in many cases, the most serious effects are manifested after a period of continuous exposure.⁴¹ The dissolved oxygen values measured immediately after the addition of MXD-100 remained high (8.1 to 8.9 mg L⁻¹), indicating that the product did not compromise the oxygenation of the medium or cause immediate oxygen consumption by organic degradation. In contrast, a study conducted by Ribeiro & Pelli,²⁴ using freshwater shrimp *Macrobrachium amazonicum*²⁴ as the target organism, observed a drop in dissolved oxygen after dilution of the product at the acute dosage, reaching a concentration of 1.93 mg L⁻¹. The other physicochemical parameters remained within ranges compatible with the survival of *Macrobrachium cf. olfersii* immediately after the addition of MXD-100.

Table 4 presents the results of the physicochemical parameters of the water in the monitored aquariums on the 38th day of the experiment, one week after the MXD-100 concentrations were diluted in the experimental units. The mortality of *Macrobrachium cf. olfersii*, observed from a concentration of 0.083 mL L⁻¹, demonstrates the acute toxic effect of MXD-100, suggesting a toxicity threshold for the species. In the study by Montresor et al.,²⁵ no toxicity was observed during 96 hours of testing with a concentration of 0.05 mg L⁻¹ of MXD-100. The chi-square analysis did not indicate significance, probably due to the small sample size and reduced number of test organisms. Since the water quality parameters remained within adequate ranges for animal survival, the mortality can be attributed to the toxicity of the compound, and not to physicochemical factors. Similar results were observed by Ribeiro & Pelli,²⁴ where the death of one individual of the freshwater shrimp *Macrobrachium amazonicum*,²⁴ out of a total of three individuals subjected to this dosage, was observed on the first day of the experiment, at the recommended dosage of 0.5 mg L⁻¹. The results of this study also demonstrated that at the acute dosage of 5.0 mg L⁻¹, all specimens died immediately after dilution of the product.

These results point to potential ecotoxicological risks of using MXD-100 in aquatic environments, even at relatively low concentrations, highlighting the need to reassess the use of this molluscicide in aquatic systems, prioritizing alternatives with less environmental impact.^{42,43}

Table 4 Physicochemical parameters of the water in the experimental aquariums and mortality of *Macrobrachium cf. olfersii* shrimp, on the 38th day of the experiment, one week after the addition of MXD-100

Parameter	Aquarium					
	1	2	3	4	5	6
Conc. MXD-100	0	0.042	0.083	0.125	0.167	0.208
Temperature	25.38	24.28	24.23	24.1	24.15	24.44
pH	7.24	7.5	7.57	7.6	7.56	7.54
Electrical conductivity	694	721	690	700	724	647
Turbidity	25.8	22.8	29.2	31.9	41.1	56.8
TDS	0.395	0.398	0.398	0.394	0.413	0.367
Chlorine	0	0	0	0	0	0
Alkalinity	180	180	180	120	180	120
Dissolved Oxygen	8.96	8.51	8.43	8.09	8.94	8.1
Mortality	0	0	1	1	1	1

Note: temperature (°C), electrical conductivity (µs/cm), turbidity (NTU), TDS (mg L⁻¹), alkalinity (mg L⁻¹ of CaCO₃), NH₃ (mg L⁻¹) and Dissolved Oxygen (mg L⁻¹)

Conclusion

The results obtained in this study demonstrated that MXD-100 has high toxicity for the two species studied: the mollusk *Physella acuta*³⁷ and the freshwater shrimp *Macrobrachium cf. olfersii* (Wiegmann, 1836). Total mortality of the mollusks was observed in all experimental units subjected to the molluscicide, starting from the lowest concentration tested, of 0.042 mL L⁻¹. For the shrimp, it was found that the concentration of 0.083 mL L⁻¹ was sufficient to cause significant mortality, establishing itself as an acute toxicity threshold for the species. The analysis of the physicochemical parameters indicated that the water conditions remained within acceptable ranges for the survival of the organisms, suggesting that the observed mortality resulted from the direct toxicity of MXD-100 and not from environmental changes. Furthermore, the reduction in dissolved oxygen levels throughout the experiments indicates the occurrence of self-purification, a process that allowed the product to degrade and enabled the organisms to survive in subsequent stages. These results reinforce the need to reassess the use of MXD-100 as a control strategy for the golden mussel in aquatic systems. Although the product is effective against fouling organisms, its ecotoxicological potential on non-target species, which are widely distributed in Brazilian ecosystems, represents a significant ecological risk. This study highlights the importance of developing and implementing less environmentally impactful alternatives capable of controlling the proliferation of the golden mussel without compromising native aquatic biodiversity.

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None.

Conflicts of interest

The author declares there is no conflict of interest.

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